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Spleen Tyrosine Kinase Confers Paclitaxel Resistance in Ovarian Cancer

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Adaptive chemoresistance and consequent tumor recurrence present major obstacles to the improvement of the prognosis and quality-of-life of patients with advanced-stage ovarian cancer. In this issue of *Cancer Cell*, Yu and colleagues describe the critical role of spleen tyrosine kinase (SYK) in paclitaxel resistance by modulating the stability of microtubules.

Paclitaxel was first introduced into clinical use during the 1990's and became part of the standard-of-care with carboplatin for the up-front treatment of advanced-stage epithelial ovarian cancer (EOC) based upon a randomized phase III trial. Despite the initial high response rate, almost all patients eventually developed platinum/taxane-resistant relapses, which resulted in minimal improvement of overall survival during the past 30 years. Even in the era of genomics, predicting paclitaxel response/resistance still remains difficult. The molecular mechanisms underlying adaptive paclitaxel resistance are also largely unknown, which is partially due to the lack of appropriate biopsy material because surgery is rarely performed in recurrent drug resistant ovarian cancers (Jayson et al., 2014).

It is well established that the microtubules are highly dynamic structures orchestrating a variety of cellular processes including cellular polarity, transportation, motility, and mitosis. Paclitaxel exerts its action by directly binding to polymerized β-tubulin, resulting in the formation of static microtubules. At therapeutic concentration, paclitaxel primarily interferes with mitotic spindle dynamics and triggers the mitotic checkpoint to induce an extended G2/M arrest that can lead to cell death via the intrinsic (mitochondrial) apoptotic pathway (Figure 1). Several general adaptive mechanisms conferring paclitaxel resistance have been identified in tumor cells, including the elevation of efflux mediated by P-glycoprotein coded by the MDR-1/ABCB1 gene, defects in spindle assembly checkpoint, mitotic slippage, activation of pro-survival signaling, and apoptosis evasion by modulating the activity of p53 and BCL2 family proteins (Murray et al., 2012). In parallel, the neoplastic microtubule system itself may also implicate in paclitaxel resistance. The aberrant overexpression of β-tubulin isotype III that lacks the predicted paclitaxel binding motif has been linked to paclitaxel resistance and poor prognosis in various tumor types, including serous ovarian cancer. However, conflicting studies suggest elevated βIII-tubulin may even sensitize cells to paclitaxel in breast cancer, clear cell ovarian cancer, and melanoma (Mariani et al., 2015). Microtubule binding proteins that modulate microtubule dynamics comprise another category of factors that may influence resistance to paclitaxel (Bhat and Setaluri, 2007). Microtubule associated proteins (MAPs), such as tau, MAP2, and MAP4, in their unphosphorylated state, promote microtubule assembly and stabilization by conformational rearrangement of tubulin subunits. MAP4 phosphorylation and dissociation from microtubules has been demonstrated to correlate with decreased paclitaxel sensitivity in paclitaxel-resistant ovarian cancer cell lines (Poruchynsky et al., 2001). Moreover, structural evidence for cooperative microtubule stabilization by paclitaxel and MAP4 has been provided by amide hydrogen/deuterium exchange coupled with mass spectrometry, further suggesting the potential relationship between MAPs and paclitaxel sensitivity (Xiao et al., 2012). Nevertheless, the precise role for MAPs in paclitaxel resistance remains to be defined.

In this issue of *Cancer Cell*, Yu et al. (2015) describe a new mechanism for paclitaxel resistance involving spleen tyrosine kinase (SYK) in high-grade advanced-stage ovarian cancer. SYK is a cytoplasmic non-receptor tyrosine kinase implicated in the physiological and pathological development of the lymphatic system and lymphoma, especially for the B cell lineage (Geahlen, 2014). Although the role of SYK in epithelial malignancy remains to be defined, elevated SYK expression has been demonstrated in recurrent epithelial ovarian tumors compared to paired primary tumors in a previous proteomics study by the same research group (Jinawath et al., 2010). Notably, tumor-specific alternative splicing of SYK triggered by EGF has been linked to mitotic spindle assembly, cytokinesis, and apoptotic evasion (Prinos et al., 2011). In this study, Yu et al. (2015) demonstrated the association of SYK and its active autophosphorylated form p-SYK at Y525/526 with chemoresistance in several independent cohorts composed of paired primary and recurrent ovarian tumors. Using primary ovarian cancer cell cultures and cell lines, the authors attributed the SYK-mediated in vitro and in vivo chemoresistance to paclitaxel rather than platinum. In various ovarian cancer cell lines with different paclitaxel sensitivities, as well as two isogenic pairs of paclitaxel-sensitive and -resistant SKOV3 and MPSC1 cell lines, the authors have convincingly demonstrated small molecule inhibitors to SYK and SYK knock-down significantly enhance paclitaxel sensitivity in various serous and clear cell ovarian cancer cell lines. Moreover, a synergistic effect of SYK inhibitor R406 (an active metabolite of

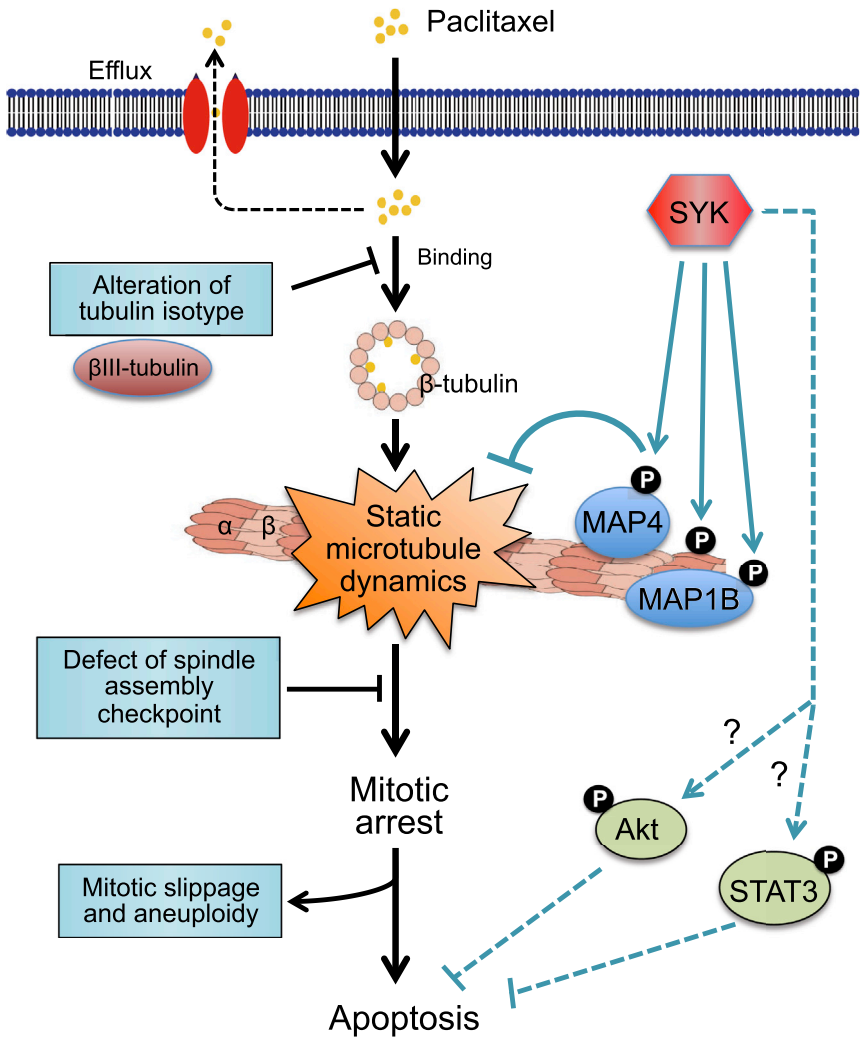

Figure 1. SYK Augments Paclitaxel Cytotoxicity by Stabilizing Microtubule Structure

Acquired paclitaxel resistance in tumor cells presents a multiplex nature. These mechanisms can be broadly categorized as microtubule dependent and microtubule independent. Tumor cells typically present higher stress tolerance to cytotoxic xenobiotics by overexpressing drug efflux proteins such as ATP binding cassette transporters. Defects in mitotic checkpoints and the apoptotic pathway also influence the resistance to paclitaxel. On the other hand, alteration of microtubule components such as tubulin isoforms (especially βIII-tubulin/TUBB3, which has widely been evident in literature) and microtubule binding proteins will directly modulate the microtubule dynamics and therefore affect paclitaxel efficacy. In paclitaxel-resistant ovarian cancer cells, elevated activation of SYK increases the phosphorylation of microtubule associated proteins MAP1B and MAP4 and attenuates their microtubule stabilizing effect. Increased microtubule dynamics is thus suggested to counteract paclitaxel. In addition, the SYK phosphoproteome analysis in the Yu et al. (2015) study also identified STAT3 and Akt as SYK downstream effectors. This observation potentially indicates the capability of SYK to induce pro-survival signaling to alleviate paclitaxel-induced stress and warrants further investigation.

fostamatinib) and paclitaxel on tumor inhibition has been observed in vitro and in vivo, especially for cells that are resistant to paclitaxel. In paclitaxel-resistant SKOV3TR cells, which do not respond to paclitaxel-induced G2/M arrest, addition of R406 with paclitaxel retards cells in the G2/M phase and potentiates cell apoptosis. Consistently, combination of R406 and paclitaxel decreases tumor growth in naive and recurrent xenograft tumor after continuous paclitaxel treatment.

Tumor cells present diverse microtubule-dependent and -independent mechanisms to develop paclitaxel resistance (Figure 1). To elucidate the molecular mechanisms by which SYK inhibition enhances the cytotoxic effects of paclitaxel, Yu et al. (2015) investigated the alteration of the phosphoproteome in SKOV3TR cells upon R406 treatment. Confirmed by the elevated levels in paclitaxel-resistant cell lines and xenografts, SYK-mediated tyrosine phosphorylation of microtubule associated proteins (MAP1B and MAP4) and α-tubulin have been suggested as the primary resistant mechanisms to counteract paclitaxel. Potentially through the restoration of the impaired binding affinity of the tyrosine phosphorylated microtubule-associated proteins, SYK inhibition has been demonstrated to directly stabilize tubulin polymers in the presence of paclitaxel in various paclitaxel resistant ovarian cancer cells in which paclitaxel alone only presents a minimal microtubule stabilizing effect. Therefore, Yu et al. (2015) have suggested a novel anti-tumor mechanism of SYK inhibition by augmenting the cytotoxicity of paclitaxel through modulating the microtubule dynamics (Figure 1).

What is exciting about this report is that several specific small molecule inhibitors of SYK, such as fostamatinib/tamatinib (R788/R406), have been developed as orally administrable and highly tolerable reagents. Their safety and efficacy have been extensively demonstrated through multiple phase I/II clinical trials to treat inflammatory and autoimmune disorders (such as rheumatoid arthritis and asthma) as well as cancer (non-Hodgkin's lymphoma and chronic lymphocytic leukemia) (Geahlen, 2014). The identification of SYK in acquired paclitaxel resistance extends the potential application of these molecules. Minimal toxicity has been observed in mice treated with R406 and paclitaxel at the effective synergistic dosages. These encouraging observations demand the clinical investigation of SYK inhibition to re-sensitize ovarian cancer relapses to paclitaxel and therefore improve the overall survival of ovarian cancer patients.

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Genetic Classification of Gliomas: Refining Histopathology

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Newly published studies validate prior reports that specific combinations of genetic alternations in *IDH1/2*, *ATRX*, *TERT*, *TP53*, and co-deletion of *1p/19q* have the ability to reclassify gliomas into rational subsets, defining a glioma's biological and clinical behavior more accurately than stratifications based solely on histopathology.

Gliomas, which include astrocytomas, oligodedrogliomas, oligoastrocytomas, and glioblastomas (GBMs), are identified and treated based on common histopathological criteria. However, these criteria may not accurately predict clinical outcome because assessment methods currently used in the clinic are overly subjective, inconsistent, and possess little power to distinguish the mixed histological appearance of glioma tissue (Olar and Sulman, 2015). Up to 43% of neuro-oncology case reviews utilizing histopathology for glial tumor identification result in some degree of disagreement, 9% of which have documented serious clinical consequences resulting from this uncertainty (Bruner et al., 1997).

Over the past decade, large-scale genetic sequencing efforts have identified key genomic alterations across glial subtypes, including mutations in *CIC*, *FUBP1*, *1p/19q* co-deletion, *IDH1/2*, *TERT*, *ATRX*, and the alternative lengthening of telomeres (ALT) phenotype. The *FUBP1* and *CIC* mutations, recently identified in oliodendrogliomas, correspond to chromosome 1p and 19q, respectively, and may represent key tumor suppressors uncovered by loss of heterozygosity (LOH) or co-deletion at 1p/19q (Bettegowda et al., 2011). Individually, each somatic alteration has demonstrated prognostic value in gliomas and other tumor types (Jenkins et al., 2006; Parsons et al., 2008; Bettegowda et al., 2011; Killela et al., 2013).

In 2012, Jiao et al. were the first to integrate these markers into a classification model across adult gliomas (Jiao et al., 2012). This initial model classified gliomas by assessing the mutation status of *IDH*, *ATRX*, and *CIC/FUBP1*, correlating the genetic information with histopathologic data and clinical outcome, and ultimately partitioned gliomas into subgroups based on shared genetic and clinical characteristics. Three subgroups emerged from this initial observation. Group 1 tumors were characterized by mutations in *IDH1* and *ATRX* and mainly demonstrated an astrocytoma phenotype on histopathology. Group 2 tumors harbored mutation in *IDH1*, *CIC*, and *FUBP1*; the majority demonstrated oligodendroglioma histology. Finally, group 3 tumors were wild-type for *IDH* and *ATRX* and were consistent with grade IV GBM histology. Patients with group 1 tumors demonstrated a median survival of 4.3 years, patients with group 2 tumors had a median survival of 8 years, and patients suffering with gliomas of the group 3 genotype had a median survival of 1.1 years.

In 2013, the discovery of promoter mutations in *TERT* in large numbers of gliomas led to a more refined classification (Killela et al., 2013). *TERT* promoter mutations were found in 83% of primary glioblastomas and were mutually exclusive with *ATRX* mutations and the ALT phenotype, which was most evident in astrocytomas (Killela et al., 2013). Furthermore, the ALT phenotype and ATRX mutations were found to be strongly linked in virtually all cases, suggesting a strong biologic correlation between disruption of ATRX and ALT. These data also provided supported the notion that telomere maintenance was an important feature of many gliomas and that two mutually exclusive